Integration of Multiple Workflow Components with Analytics in SuraSeq® Targeted Assays Improves Mutation Accuracy in Residual Clinical FFPE and FNA Biopsies

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SUMMARY

- NGS is a critical tool for oncology research and diagnostics, but difficult to implement due to wet lab and analysis burdens that are further complicated by tumor heterogeneity.
- The SuraSeq® Targeted NGS Assay optimizes the analysis of low-quality FFPE and low-quantity FFPE and FNA samples by measuring the number of functional DNA copies prior to library prep using the Quantidex™ DNA Assay.
- NGS data from 474 samples, including 171 FFPE and 254 FNA specimens, were used to develop a variant classifier that incorporates functional DNA copy input information.
- The Quantidex™ variant caller links pre-analytical, analytical, and post-analytical steps to enable superior variant call performance compared to other methods and improves the fraction of challenging specimens that can be accurately analyzed.

INTRODUCTION

Utilization of next-generation sequencing (NGS) in clinical research and diagnostics is rapidly growing. However, complexities inherent to the experimental workflow and analysis pose significant challenges to NGS adoption. We describe SuraSeq® Targeted NGS Assays, which utilize integrated and platform-neutral workflows, reagents, and analyses to reduce sample input requirements to as low as 100 amplifiable copies (<1 ng) of DNA while retaining high sensitivity and PPV. We present a variant calling algorithm that incorporates Quantidex™ Assay-derived functional DNA assessments and improves accuracy on clinical specimens.

MATERIALS AND METHODS

DNA functionality was assessed by the Quantidex™ DNA Assay (adapted from Sah et al., 2013), and PCR-based target enrichment was conducted using SuraSeq® NGS reagents (modified from Hadd et al., 2013). Samples were comprised of cancer cell-line DNA mixtures (8), HapMap samples (2), synthetic gBlock constructs spiked into genomic DNA (2), and a total of 462 clinical FFPE (171), FNA (254), Plasma (18), and fresh-frozen (19) specimens obtained under IRB approval. Sequencing procedures for MiSeq (Illumina) and PGM (Thermo Fisher) followed manufacturer's instructions. Mutational status was determined by sequencing with verification by liquid bead array (Luminex) (333) and/or replicate sequencing (467) and considering concordant calls positive after accounting for site and sample-specific background. Sequencing analysis was performed by Asuragen's bioinformatics pipeline. Variant calling using VarScan2 (Koboldt et al., 2012) was performed in accordance with recommended protocols (Koboldt, et al. 2013).

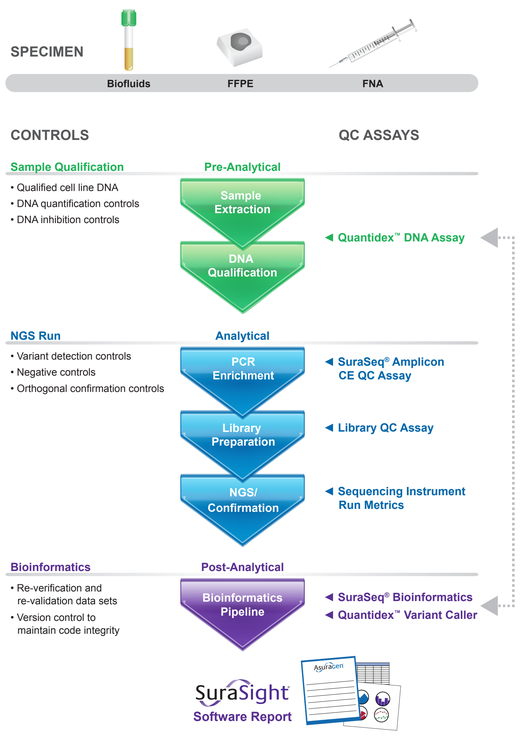


Figure 1. Workflow for SuraSeq® targeted NGS.

Presented at AGBT 2015

RESULTS

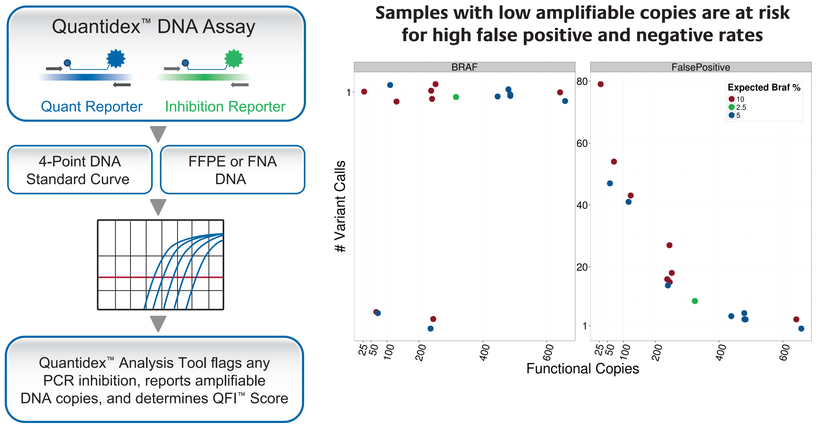


Figure 2. Quantification of amplifiable DNA by the Quantidex™ DNA Assay guides input into the NGS enrichment step to help ensure the accuracy of variant calling. A) Quantidex™ Assay workflow, **B)** Low functional copies put samples at risk for high false positive and high false negative rates.

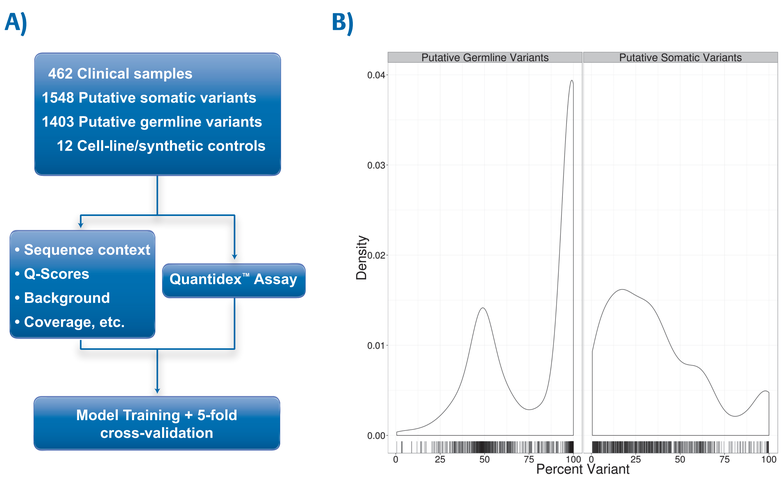
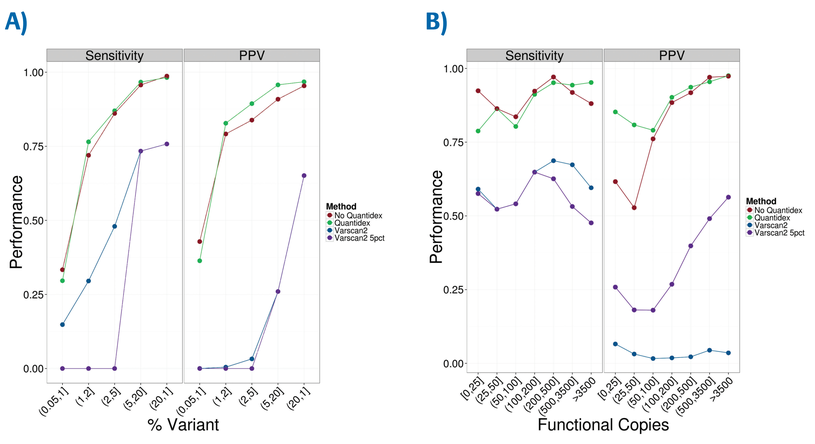


Figure 3. Strategy and experimental design used to assess the utility of incorporating Quantidex™ Assay results directly into the variant caller. A) Samples and design for training a classifier with and without Quantidex™ Assay data. **B)** Positive variant data binned into putative germline variants shows a characteristic bimodal allele frequency distribution. Putative somatic variants demonstrate a skew to lower abundance variants. Taken together, the data suggest a reasonable approximation of somatic vs. germline variants.



C)

Sensitivity	PPV	Method	
		Variant Caller	Parameters
0.99	0.98	Asuragen	No Quantidex™ Assay
0.99	0.99	Asuragen	Quantidex™ Assay
0.51	0.88	Varscan2	>5% Variant Threshold
0.51	0.31	Varscan2	Overall

Figure 4. Incorporation of Quantidex™ Assay results into the bioinformatic analysis improves the sensitivity and reliability of variant calls. Sensitivity and PPV are shown for putative somatic variants in sample bins of **A)** percent variant allele and **B)** functional copy numbers. The Quantidex™-enabled caller is cautious in calling low-copy DNA samples and more aggressive for high-copy samples. **C)** Performance with putative germline variants.

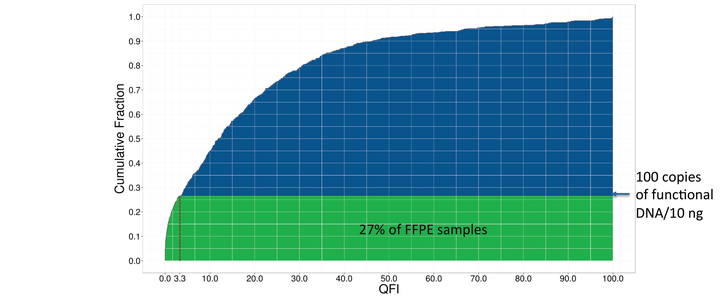


Figure 5. The Quantidex™ variant caller increases the fraction of clinical samples that can be reliably sequenced. Shown is the empirical cumulative distribution of QFI for a cohort of 664 clinical FFPE samples. A total of 27% of samples fell below 100 functional copies per 10 ng of bulk (A260) DNA input and are at increased risk for high false positive and false negative rates. The Quantidex™ caller lowers the risk of false positive calls for this group of samples.

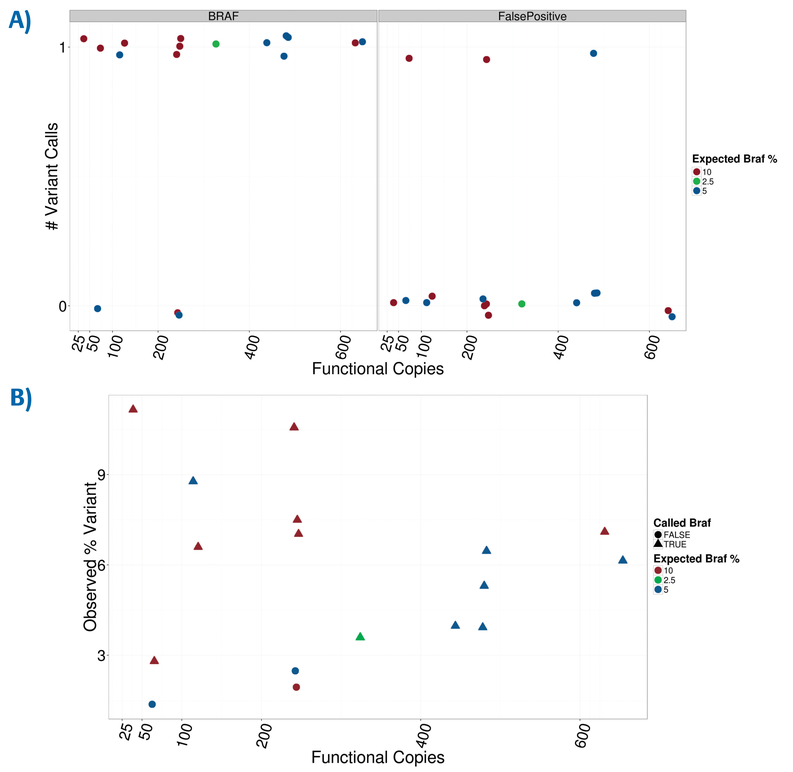


Figure 6. BRAF V600E case study: Accurate variant detection with low-quantity, low-quality residual clinical FFPE DNA. A BRAF V600E-positive FFPE was titrated into the background of a BRAF wild-type FFPE sample to 2.5% variant. Functional copies were titrated between 30 and 660. The samples were called with the trained Quantidex™ model. A) Total number of variant calls. Points are colored by theoretical BRAF % and have been jittered to avoid over-plotting. B) Observed variant allele frequency vs. functional copy input. Points are colored by theoretical BRAF % and shaped according to BRAF-called (triangles) or not (circles). The Quantidex™ caller maintained high sensitivity and PPV, even at low copy inputs and low % variant.

CONCLUSIONS

- The incorporation of Quantidex™ DNA Assay results increases the PPV of variant calling by 51% at the lowest copy-number inputs with only an 8% decrease in sensitivity.
- The ability to call variants in low-quality and low-quantity DNA samples increases the number of clinical samples that can be processed with high confidence. In our cohort, more than a quarter of samples fell into this category.
- The trained Quantidex™ model called BRAF variants in residual clinical FFPE with as few as 34 and 70 functional copies of input, representing just 3.74 (11% variant) and 1.96 (2.8% variant) mutant copies, respectively.
- Use of bioinformatic approaches that incorporate sample-specific quality control data increases the number of clinical samples that can be accurately assessed.